Utility of Chromosomal Chromogenic in Situ Hybridization as an Alternative to Flow Cytometry and Cytogenetics in the Diagnosis of Early Partial Hydatidiform Moles

A Validation Study

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OBJECTIVE: The introduction of p57 immunohistochemistry has aided the distinction between early complete moles (CMs) and hydropic abortus (HA), but no single technique has emerged for the distinction between early partial moles (PMs) and HA. Flow cytometry and cytogenetics have been used, but these require specialized equipment/expertise. The goal of this study is validation of chromosome in situ hybridization (CrISH), focusing on comparing the results to those obtained by cytogenetic methods.

STUDY DESIGN: Archival paraffin blocks from molar and nonmolar gestations were retrieved. Sections were labeled with a chromosome 10 probe. Hybridization and visualization were performed using standard protocols. One hundred nuclei per sample were scored for the number of signals.

RESULTS: Of 50 hydatidiform moles, 22 were PMs and 28 were CMs. The CMs showed 2 signals in 25 cases and 4 signals in 3 cases. The PMs showed 3 signals in 21 cases and 2 signals in 1 case. For the HAs there were 2 signals in 24 cases, and 1 case had 3 signals. Concordance between CrISH and flow cytometry studies for molar gestations was 95%.

CONCLUSION: CrISH is a highly effective adjunct in differentiating between PM and CM and between PM and HA. CrISH is a simple, cost effective adjunct in evaluating molar gestations. (J Reprod Med 2010;55:275–278)

No single diagnostic tool has emerged to allow for the unequivocal diagnosis of partial hydatidiform moles.

Keywords: chromosome, flow cytometry, hybridization, hydatidiform mole, ploidy.

The distinction between complete and partial hydatidiform molar gestations and between those entities and hydropic abortuses (HAs) has important

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patient management implications.[1-3] This is becoming increasingly true in the setting of an increasing use of assisted reproductive technologies, where patients are waiting until later in reproductive life to achieve pregnancies. Traditionally, conventional histologic evaluation of standard H-E sections was generally sufficient to distinguish between hydatidiform moles (both complete and partial) and HAs. The majority of pathologic specimens were collected after 16 weeks' gestational age, by which point the significant histologic features of complete and partial moles were typically well-developed. However, during the last few decades, the availability of high-quality ultrasound imaging has led to increasingly early sampling of intrauterine gestations, such that most samples are now obtained before 10 weeks' gestational age.[4,5] This has led to somewhat of a paradigm shift as new sets of histologic criteria for "early" complete and partial moles (CMs and PMs) have been necessitated to reflect this new type of specimen.[6,7] The features of early CMs have become relatively well-defined and include hypercellular, mucoid villous stroma (rather than the markedly hypocellular, cavitated villi seen in well-developed complete hydatidiform moles), villi having irregular contours with scalloped edges and polypoid projections (often described as "budding villi") and significantly increased stromal karyorrhexis.[7] In contrast, the features of so-called early partial hydatidiform moles are much less well-established, probably owing to the more focal nature of histologic changes seen even in well-developed PMs as compared to CMs.[8] Thus, the distinction between early complete hydatidiform moles and early partial hydatidiform moles and between early partial hydatidiform moles and HAs can be challenging.[9]

The introduction of immunohistochemical analysis of $p57^{kip2}$ expression has virtually revolutionized the way in which molar gestations are diagnosed.[9-11] $p57^{kip2}$ is a paternally imprinted gene located on chromosome 11. Due to the imprinting, there is expression only from the maternal genome. Because complete hydatidiform molar gestations are androgenetic, there is a loss of the $p57^{kip2}$ protein in villous cytotrophoblasts, whereas in partial hydatidiform moles and in HAs (which both have biparental DNA contributions), the $p57^{kip2}$ protein is retained in these cells. For reasons that are not well understood, even in complete moles, $p57^{kip2}$ expression is retained in extravillous trophoblasts, which provides an excellent internal control for the immunohistochemical assay.[9] Thus, complete hydatidiform moles—even at very early stages of development—can be diagnosed unambiguously through the use of $p57^{kip2}$ immunohistochemistry. No single diagnostic tool has emerged to allow for the unequivocal diagnosis of partial hydatidiform moles; however, the use of DNA ploidy analysis has shown itself to be a very helpful adjunct in making the distinction between a diploid CM and a triploid PM, and it may be useful in aiding in the distinction between a diploid HA and a triploid PM.[8,9,12] Numerous techniques currently exist for evaluating DNA ploidy, including flow cytometry, fluorescence in situ hybridization, cytogenetic analysis and chromogenic chromosomal in situ hybridization (CrISH).[9,13,14] All but the last of these suffer from the requirement for expensive equipment, highly specialized training and/or the need for fresh tissue. CrISH provides a means to circumvent many of these issues. It can be performed on formalin-fixed, paraffin-embedded tissue, which is the standard in most pathology laboratories.[13] The reagents used are similar to those needed for routine immunohistochemistry. Finally, the overall costs of performing CrISH are significantly lower than those for conventional cytogenetic, flow cytometry or fluorescence-based methods. The goal of the current study is to validate CrISH with a focus on comparing the results to those obtained by cytogenetic methods.

Materials and Methods

For this study, which was approved by the Institutional Review Board at Partners Healthcare, 50 cases of hydatidiform molar gestation and 25 cases of nonmolar HA were identified through the pathology database at Brigham and Women's Hospital. All cases used for the study had cytogenetic or flow cytometric analyses as part of the work-up. Paraffin blocks were obtained from the archives and 5 µm sections were cut for each case. The sections were mounted on poly-L-lysine–coated slides and deparaffinized according to standard protocols. Slides were incubated at 50°C with proteinase K (500 mm/mL) and 0.1% Triton X-100 for 60 minutes. Following rinsing, dehydration and air-drying, the biotinylated DNA probe (Stratagene) for chromosome-10 was added to achieve a concentration of 1 ng/mL. Denaturation was carried out at 90°C for 15 minutes, followed by overnight hybridization at 37°C. Slides were rinsed in 2× SSC (salt, sodium citrate) with 50% formamide at 37°C.

Color development was carried out using avidin, biotinylated mouse-anti-avidin, rabbit-anti-mouse peroxidase, and 3,3'-diaminobenzidine according to standard protocols. Finally, slides were counterstained with hematoxylin and coverslipped. Each case was evaluated independently by 2 pathologists (A.F. and D.K.) in a blinded fashion. Using standard light microscopy, the number of signals was counted in each of 100 nonoverlapping villous stromal cell nuclei (at 400× magnification).

Results

Figure 1 shows examples of CrISH in diploid (A) and triploid (B) nuclei. Of the 50 cases of hydatidiform moles 22 were diagnosed as PMs and 28 were diagnosed as CMs. In 25 of the CMs ploidy studies revealed diploid DNA content, and in 3 a tetraploid DNA content was observed. There was 100% concordance between the CrISH results and the ploidy studies in the CM cases. Additionally, there was 100% agreement between the 2 pathologists when evaluating the CrISH cases. Concerning the PMs, 20 cases (with triploid DNA content by ploidy studies) were found to have 3 signals when evaluated by CrISH, and 2 cases had 2 signals. For the 25 HAs 24 revealed 2 signals when evaluated by CrISH, and 1 showed 3 signals. Overall, the concordance between CrISH and other ploidy studies was 95%.

Discussion

Distinguishing between partial hydatidiform moles and HAs (particularly specimens at early gestational ages) presents one of the more difficult diagnostic challenges facing practicing pathologists. No single test or set of features has emerged to allow for easy resolution of this diagnostic dilemma. The use of immunohistochemistry for $p57^{kip2}$ has made the identification of complete hydatidiform moles relatively straightforward, but this adjunctive test is not useful in differentiating between PMs and HAs due to the biparental DNA contributions in both situations. Ploidy studies have emerged as the most useful ancillary test, owing to the fact that the vast majority of partial hydatidiform moles will possess a triploid DNA content while HAs should have diploid DNA content (or have alterations in individual chromosome complement, such as trisomies). Several methods exist for the evaluation of ploidy, including flow cytometry, cytogenetics, fluorescence in situ hybridization and CrISH. The current study shows a high concordance rate (95%) between CrISH and flow cytometry/cytogenetics in a large number of cases (both molar gestations and HAs). The single case of the HA that showed 3 signals when evaluated by CrISH may be an example of a trisomy at chromosome-10 and illustrates one of the drawbacks of using only a single chromosome probe. Others have advocated using a cocktail of chromosome probes, however, the use of a single DNA probe (to chromosome-10 in this study) minimizes the costs of performing this assay considerably, such that it is much less expensive than cytogenetics, flow cytometry and fluorescence-based methods (with their relatively high costs for specialized equipment, reagents and technical expertise). It is critical to emphasize the importance of the histologic findings in evaluating early gestations. One of the major drawbacks of flow cytometry (in addition to the high initial cost of setting up the required instrumentation) is the complete loss of morphologic correlations. We present data suggesting that CrISH using a single chromosome probe is a highly effective adjunct in differentiating between partial and complete hydatidiform moles and between PMs and HAs. Used in combination with $p57^{kip2}$ immunohistochemistry and morphology,

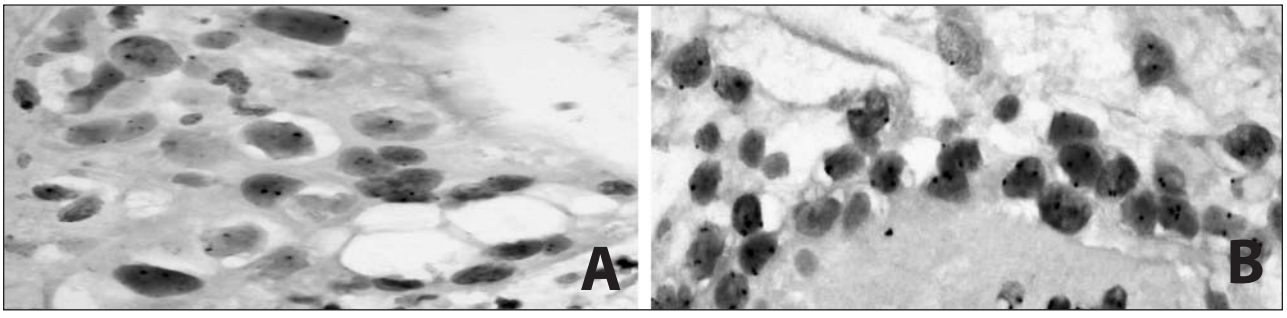

Figure 1 Example of CrISH in a case with diploid DNA content (A) and in a case with triploid DNA content (B).

CrISH provides a simple, cost-effective adjunct to help subclassify molar gestations.

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